In Silico Studies of Fenugreek Seeds' Phytochemicals Anti-Aging Compounds

Adeyemi, Olanrewaju Sanjo [1,2,]*, Kehinde, Ibrahim Oluwatobi [3,] Abiola, Julianah Ore [1,2,] Omotuyi, Idowu Olaposi [2,3] Oyinloye, Babatunji Emmanuel [1,2]

1Biochemistry Programme, Department of Chemical Sciences, College of Sciences, Afe Babalola University, Ado-Ekiti, Nigeria;
2Institute of Drug Research and Development, SE Bogoro Center, Afe Babalola University, Ado-Ekiti, Nigeria;
3Department of Pharmaceutical Science, Faculty of Pharmacy, Afe Babalola University, Ado-Ekiti, Nigeria

Corresponding Author: Adeyemi

Abstract: *Ageing is the accelerated loss of forms and functions of cellular components. Pathological ageing affects every human. AMP-activated protein kinase (AMPK) and Mammalian target of rapamycin (mTOR) mTOR proteins play major roles in maintaining cellular energy homeostasis by noticing and responding to AMP/ATP level and nutrients for protein synthesis. AMPK and mTOR have been implicated in various spectrum of aging-associated diseases like cancer, diabetes and cardiovascular diseases. Inhibition of mToR protein has been engaged in the treatment of diseases by targeting the P13k/AKT pathway while the activation of AMPK is believed to extend lives. This computational approach compares phytochemicals from T. foenum in activation of AMPK to a standard drug (segluromet) in clinical trial and the inhibitory capacity of T. foenum on MToRC1 complex through the FK506 Binding Protein binary complex which interact with the mToR protein, also compared with SAFIT2 and rapamycin which inhibit mTOR to bring about autophapy or inhibition of protein synthesis and fat accumulation in the body. Our result shows that T foenum phytochemicals could be considered as potential AMPK activators and Inhibitors of mTOR pathway. Hence, we can recommend it for in vivo study.*

Keywords: *Ageing, AMPK, mTOR Protein, T. foenum, Seglurome.*

1. Introduction

Ageing is a progressive deterioration of cellular components that finally leads to loss of functions or the death of the cell. The human body is made up of millions of cells. The death of cells leads to the non-functionality of organs, tissues, the entire organisms. (Adams and White, 2004, Rastogi *et al*., 2015).

Causes of ageing have been grouped into three categories: Environmental pollutants from industrial waste and smokes. Dietary source of ageing includes excess sugar and foods which undergo autooxidation and other reactions to generate free radicals or toxic chemicals that can initiate ageing and finally the natural cause of ageing which occur at the genomic level (Eaknai *et al*.,2022). These causes have led to the propagation of over three hundred theories of ageing which include: Telomere theory, loss of functions theory, genomic instability, endocrine theory, stem cell exhaustion theory, mitochondrial dysfunction theory, wear and tear theory and the free radical damage theory (Rastogi *et al*., 2015). The widely accepted theory is the free radical theory because it serves as foundation to explain all other explainable theory of ageing Harman,1992).

AMP-activated protein kinase (AMPK) performs a major role in balancing the cellular energy homeostasis by sensing and responding to AMP/ADP levels relative to ATP (Carling *et al*.,

2011). AMPK is a serine/threonine kinase. It is taxonomically conserved in all eukaryotes and widely expressed as a heterotrimeric complex. It is structurally comprised of a catalytic α subunit, a regulatory γ subunit with a scaffolding β subunit. (Liu *et al*., 2014). AMPK manages multiple input signals indirectly/directly with limited energy availability to coordinate various energy-saving responses in the whole body (Viollet *et al*., 2014). When the body performs any task, ATP is converted to AMP or ADP, leading to a high concentration of AMP, which allosterically activates AMPK. When AMPK is activated, energy production is initiated by activating glycolysis and glucose transport, which enhances the conversion of AMP to ATP (Carling, 2004.) and these ensure the systematic balancing of the cellular energy requirement. AMPK is implicated as a target in cancer as a typical energy-related pathological intervention (Xu and Si, 2010). Hence, allosteric activation of AMPK would sustain cellular functionality and extend life span. (Yu *et al*.,2021).

The mammalian target of rapamycin (mTOR) – a typical serine/threonine (S/T) protein kinase, is also another central controller of proliferation and cell growth which controls cellular metabolic processes owing to its unique ability to sense nutrients (Lamming, 2016). Abnormal activation of the mTOR pathway was detected in adenocarcinomas as well as bronchioloalveolar

carcinomas (Hiramatsu *et al*.,2010). During inhibition, rapamycin forms complex with the intracellular receptor FKBP12 (Sabers *et al*.,1995), this complex binds to mTOR and inhibits mTORC1 downstream signaling, thereby preventing translations of the proteins involved in progression of cancer cells (Ma and Blenis, 2009). The present challenge is to identify a better inhibitor with better affinity and lesser or no side effect that could replace rapamycin (Morath *et al*.,2007).

The mammalian target of rapamycin (mTOR) – a typical serine/threonine (S/T) protein kinase, is also another central controller of proliferation and cell growth which controls cellular metabolic processes owing to its unique ability to sense nutrients (Lamming, 2016). Abnormal activation of the mTOR pathway was detected in adenocarcinomas as well as bronchioloalveolar carcinomas (Hiramatsu *et al*.,2010). During inhibition, rapamycin forms complex with the intracellular receptor FKBP12 (Sabers *et al*.,1995), this complex binds to mTOR and inhibits mTORC1 downstream signaling, thereby preventing translations of the proteins involved in progression of cancer cells (Ma and Blenis, 2009). The present challenge is to identify a better inhibitor with better affinity and lesser or no side effect that could replace rapamycin (Morath *et al*.,2007).

2 Materials and Methods

2.1 Ligand preparation

Structures of *T. foenum* phytochemicals were fetched from PUBCHEM database in 3D (SDF) format. The standard drug for activating AMPK segluromet (Metformin hydrochloride) with higher binding score than metformin was obtained from PubChem. Rapamycin and SAFIT2 were also downloaded from the PUBCHEM databank as standard ligands for mTOR.

2.2. Proteins preparation

Structure of AMPk (PDB ID: 4AE1) was obtained from the protein databank. A crystal structure of mTOR (PDB ID: 4JSV) was downloaded from the Protein Data Bank (PDB). FKBP (PDB

ID: 4TW6) was also downloaded.

2.3. Molecular Docking process:

Autodock vina was used for the docking process. The grid was generated to determine the binding site on the proteins. ADMETOX analysis and Lipinski rule of 5 were carried out to determine the drug likeliness of these molecules.

3. Docking result

3.1 Result: Activation of AMPK

3.1.1 Docking Scores of Most Active Phytochemicals in Fenugreek Against the AMP Activated Protein Kinase as The Target Using Autodock vina tools:

After ligands and protein preparation process with the autodock vina, the sixty-six (66) ligands were docked against the AMPK (PDB number 4EAI). Activation of AMPK is theoretically believed to delay ageing process. The binding scores were reported below in comparison with the standard drug called segluromet.

Table 3.1: Docking scores of most active phytochemicals in fenugreek against the Adenosine monophosphate activated Protein kinase (AMPK) as the target using the Autovina docking tool

BIOACTIVE COMPOUND	4EA1 (BINDING SCORES)
Yamogenin	-9.42
Spirostanol	-9.40
Diosgenin	-9.28
Gitogenin	-9.27
Tigogenin	-9.22
Rutin	-9.02
Neotitogenin	-9.01
Segluromet (Standard drug)	-5.712

After the docking of all the ligands with the target protein, seven ligands with higher binding affinity than the standard drug (segluromet) were reported as shown in the table 3. 1 above.

3.1.2 The Evaluation of Oral Drug-Likeness of The Best Seven Compounds that Activate AMPK Using Lipinsky Rule of Five

Lipinski rule of five was used to predict the drug-likeness of the bioactive components in T .foenum. The permeability of molecules across membrane according to Lipinsky should be less than or equal 500g/mol. The lipophilicity of the molecules which also affect the permeability across the non-polar membrane determined by the hydrogen bond interaction which is a function of hydrogen bond donation and hydrogen bond acceptance and the number of free rotating bond. The partition co-efficient is also a function of the compound's solubility in liquid or gaseous medium. According to Lipinski rule, a good candidate for drug should have a Log P< 5 or the MlogP< 4.15, HBA<10, HBD< 5, free rotatable bond< 5 and Mol weight< 500gram.Potential drug candidate should not fail more than two of the five rules. Table 4.2 shows the result using molsoft package(wwww.molsoft.com).

Table 3.2: The Evaluation of Oral Drug-Likeness of The Best Seven Compounds Using Lipinsky Rule of Five

ENTRY NAME	MOLECULAR WEIGHT	HYDROGEN BOND DONOR	HYDROGEN BOND ACCEPTOR`	MLog P	ROTATIONAL BOND	LIPINSKI VIOLATION/ FAIL
Yamogenin	414.62	1	3	4.94	0	1
Spirostanol	416..64	1	3	5.08	0	1
Diosgenin	414.62	1	3	4.94	0	1
Gitogenin	432	2	4	4.23	0	1
Tigogenin	416.64	1	3`	5.0`	0	1
Rutin	610	10	16	-3.89	6	4
Neotitogenin	432	2	4	4.23	0	1

3.1.3 ADME/TOX Prediction of Selected Compounds that Activate AMPK.

Table 3.3

ENTRY NAME	MOLECULAR WEIGHT	BLOOD BRAIN BARRIER (PERMEABILITY)	GI ABSORPTION`	CYP1A2 INHIBITOR	CYP2C19 INHIBITOR	CYP2C9 INHIBITOR	CY2D6 INHIBITOR
Yamogenin	414.62	YES	HIGH	NO	NO	NO	NO
Spirostanol	416.64	YES	HIGH	NO	NO	NO	NO
Diosgenin	414.62	YES	HIGH	NO	NO	NO	NO
Gitogenin	432	YES	HIGH	NO	NO	NO	NO
Tigogenin	416.64	YES	HIGH	NO	NO	NO	NO
Rutin	610	NO	LOW	NO	NO	NO	NO
Neogitogenin	432.64	YES	HIGH	NO	NO	NO	NO

3.1.4 3D Protein-Ligand Interactions Between the seven (ligands) and AMPK

3.1.4.1 Yamogenin and AMPK interaction.
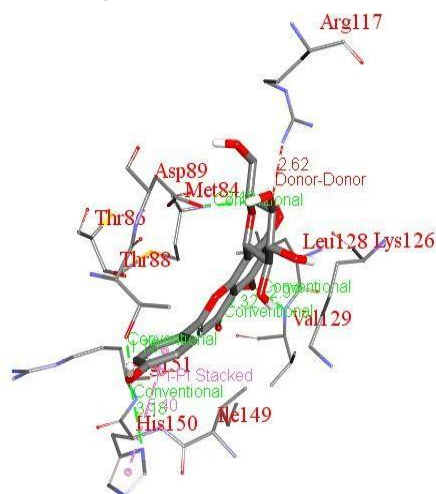

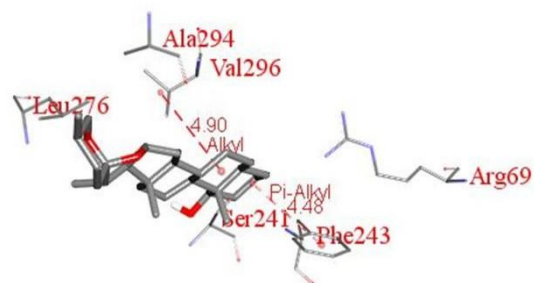**Figure 3.1:** Yamogenin and AMPK

3.1.4.2 Spirostanol and AMPK

Figure 3.2 Spirostanol and AMPK
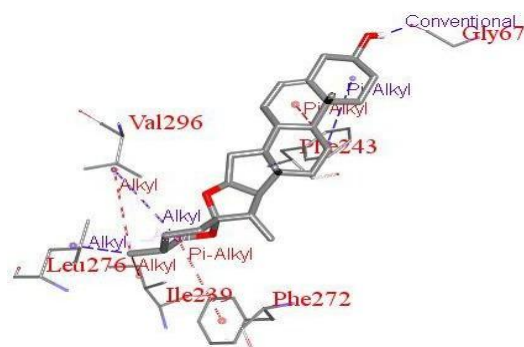
3.1.4.3 Diosgenin and AMPK

Figure 3.3: Diosgenin and AMPK

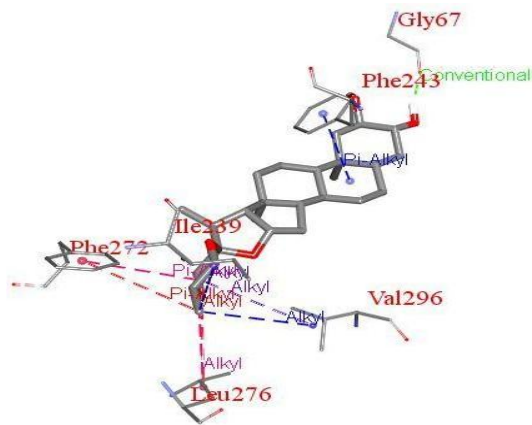**3.1.4.4** Gitogenin and AMPK

Figure 3.4: Gitogenin and AMPK

3.1.4.5 Tigogenin and AMPK as illustrated by Figure 4.5
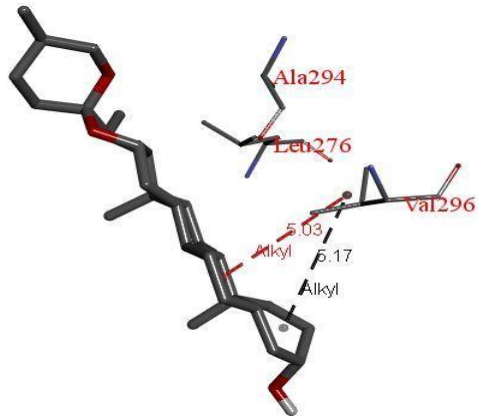

Figure 3.5: Tigogenin and AMPK
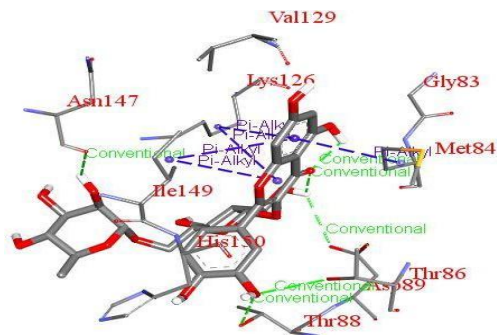
3.1.4.6 Rutin and AMPK as illustrated by   Figure 4.6

Figure 3.6: Rutin and AMPK

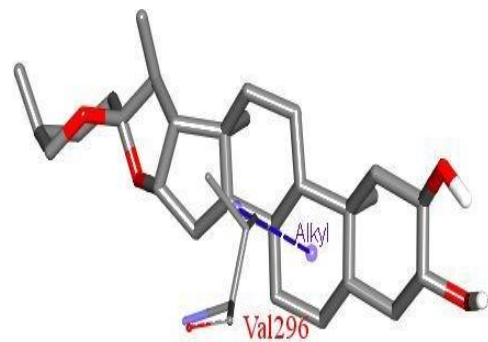**3.1.4.7** Neogitogenin and AMPK as illustrated by Figure 4.7

Figure 3.7: Neogitogenin and AMPK

3.2.0 INIHIBITION OF MTOR AND FKBPs

3.2.1. Docking Result: The inhibition of mTOR protein is normally achieved when ligand binds with FK506-Binding Protein to form a binary complex and the   FKB-Rapamycin complex binds with mToR protein. The Ligands normally dock to the domain of FK BP

Table 3.4: showing the docking scores of *T.Foenum* phytochemicals with mTOR protein PDB 4JSV

BIOACTIVE COMPOUNDS	4JSV PROTEIN (BINDING SCORE)
Carpaine	-11.0
1-Spirostanol	-10.38
Yuccagenin	-10.14
Sarsasapogenin	-10.34
Tigogenin	-10-20
Diosgenin	10.12
Smilagenin	-10.03
Rapamycin	-10.78
Cocrystalised Ligand (ADP)	-7.34

Table 3 .5: showing the docking scores of *T. Foenum* phytochemicals with mTOR protein PDB 4JSV

FKBP51(1KT0) With a FKBD-1(Binding domain 4TW6)

BIOACTIVE COMPOUND	4TW6 BINDING SCORE
1-Spirostanol	-8.88
Gitogenin	8.83
Neotitogenin	8.60
Sarsapogenin	8.55
Diosgenin	7.94
Safit 2	7.06
Cocrystalised Ligand (FIT1)	-6.6

3.2.2 The Evaluation of Oral Drug-Likeness of The Best Ten Compounds Using Lipinsky Rule of Five

Table 3.6 The Evaluation of Oral Drug-Likeness of The Best Ten Compounds Using Lipinsky Rule of Five

ENTRY NAME	MOLECULAR WEIGHT	HYDROGEN BOND DONOR	HYDROGEN BOND ACCEPTOR	MLogP	ROTATIONAL BOND	LIPINSKI VIOLATION/FAIL
Carpaine	478	2	6	3.75	0	0
1-Spirostanol	416..64	1	3	5.08	0	1
Yuccagenin	430,60	2	4	4.09	0	0
Sarsasapogenin	416.64	1	3	5.08	0	1
Tigogenin	416.64	1	3`	5.0`8	0	1
Diosgenin	414.62	1	3	4.94	0	1
Smilagenin	416.64	1	3	5.08	0	1
Gitogenin	432	2	4	4.23	0	1
Neotitogenin	432	2	4	4.23	0	1

3.2.3 ADME/TOX Prediction of Selected Compounds that inhibited mTOR and 4TW6

Table 3.7 ADME/TOX Prediction of Selected Compounds that inhibited mTOR and 4TW6

ENTRY NAME	MOLECULAR WEIGHT(G/MOL)	BLOOD BRAIN BARRIER (PERMEABILITY)	GI ABSORPTION`	CYP1A2 INHIBITOR	CYP2C19 INHIBITOR	CYP2C9 INHIBITOR	CY2D6 INHIBITOR
Carpaine	478.0	NO	HIGH	NO	NO	NO	NO
1-Spirostanol	416.64	YES	HIGH	NO	NO	NO	NO
Yuccagenin	430.62	YES	HIGH	NO	NO	NO	NO
Sarsasapogenin	416.64	YES	HIGH	NO	NO	NO	NO
Tigogenin	416.64	YES	HIGH	NO	NO	NO	NO
Diosgenin	414.62	YES	HIGH	NO	NO	NO	NO
Smilagenin	416.64	YES	HIGH	NO	NO	NO	NO
Gitogenin	432	YES	HIGH	NO	NO	NO	NO
Neotitogenin	432.64	YES	HIGH	NO	NO	NO	NO

3.2.4 Receptor-Ligands Interaction

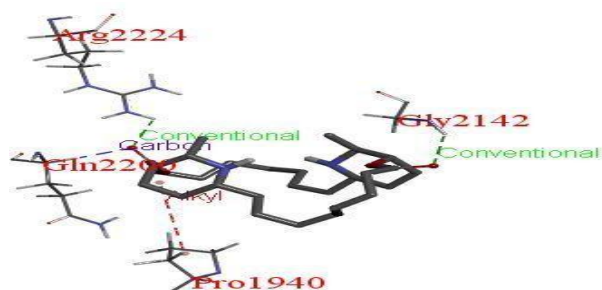**3.2.4.1 Carpaine Interaction with 4JSV**

Figure 3.8: Carpaine Interaction with 4JSV
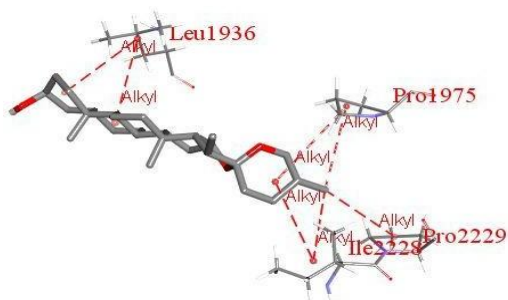
3.2.4.2 Tigogenin interaction with 4JSV

Figure 3.9 Tigogenin interaction with 4JSV

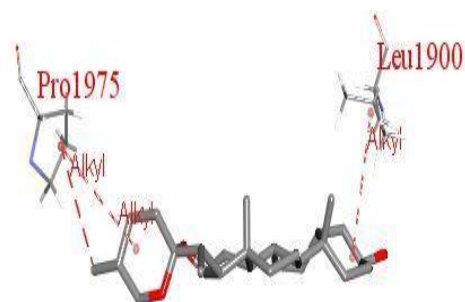**3.2.4.2 Smilagenin Interaction with 4JSV**

Figure 3. 10 Smilagenin interaction with 4JSV

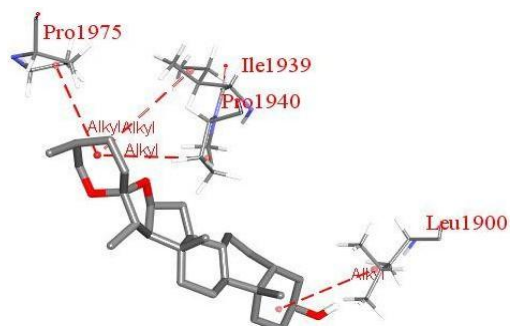**3.2.4.3** Sarsasapogenin interaction with 4JSV

Figure 3.11: Sarsasapogenin interaction with 4JSV
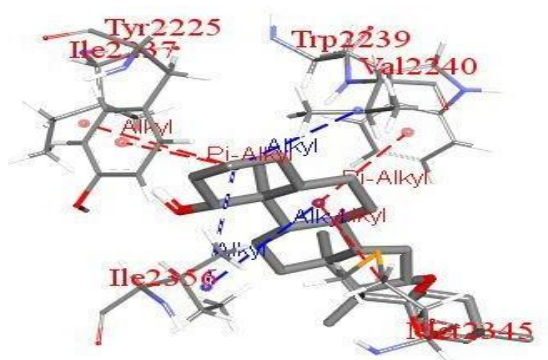
3.2.4.6-Spirostanol interaction with 4JSV
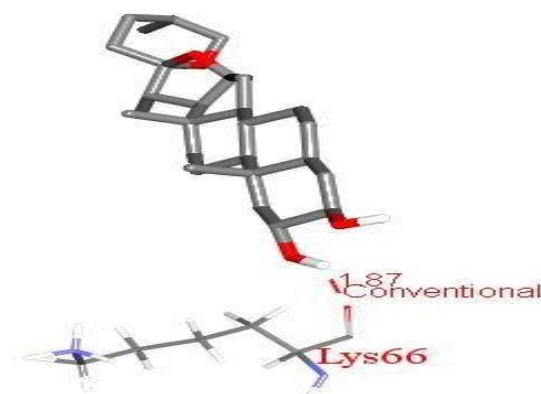
Figure 3. 12 Spirostanol interaction with 4JSV

3.2.4.7 Diosgenin interaction with 4JSV

Figure 3.13: Diosgenin interaction with 4JSV

3.2.4.8 Gitogenin interaction with 4TW6

Figure 3.14 Gitogenin interaction with 4TW6

3.2.4.9 Neotitogenin interaction with 4TW6

Figure 3.15 Neotitogenin interaction with 4TW6

3.2.4.10 Sarsasapogenin interaction with 4TW6

Figure 3.16: Sarsasapogenin interaction with 4TW6

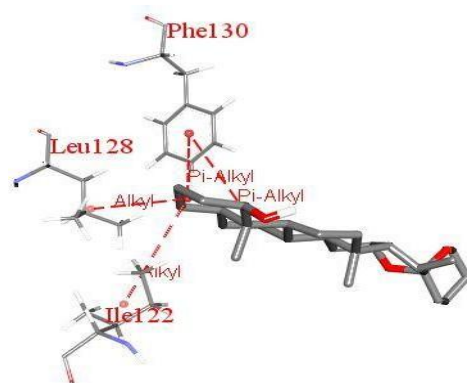**3.2.4.11** Spirostanol interaction with 4TW6

Figure 3.16 Spirostanol interaction with 4TW6

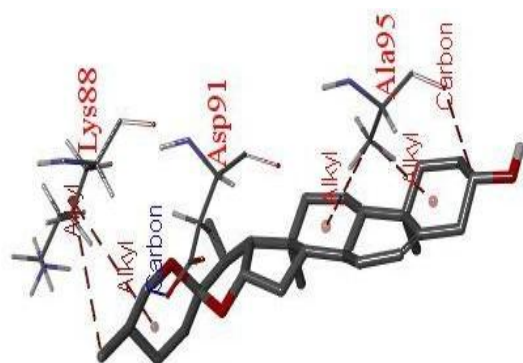**3.2.4.12** Diosgenin interaction with 4TW6

Figure 3.17 Diosgenin interaction with 4TW6

5. Discussion

Biological ageing can be delayed by activation of AMPK which induces autophagy (Adams & White, 2004). From Table 3. 1, Segluromet which has been by adopted for delaying ageing process has a binding score of -5.71 while yamogenin, spirostanol, diosgenin, gitogenin, tigogenin, rutin and neotitogenin have better binding affinity than segluromet. Yamogenin tops the list with binding score of -9.42. Hence, these phytochemicals could be responsible for anti-ageing property of T. *foenum* as reported locally by ancient tradition.

From result in table 3.2, all the ligands passed the Lipinski rule except rutin that violates 4 out of the 5 rules which shows that rutin could not be a good candidate for drug despite the fact that it has better binding score than segluromet in table 3.1.

Using swissADME package, we could report that rutin had molecular mass greater than 500g and could not cross the blood-brain barrier. Also, except rutin, others have higher value in term of gastro-intestinal absorption. This is illustrated in table 3.3.

The ligand-protein interaction shows the stability and degrees of interaction at the active site. Between AMPK active site and Yamogenin, amino acids like ARG [117], LEU [28], LYS [126], VAL [29], MET [49], HIS[150], THR [88], THR [86] interacted with ligand showing various conventional hydrogen bonding and Pi-Pi stacking.

AMPK and Spirostanol shows Ligand -Alkyl interaction with PHE [243] and other conventional non covalent interaction with amino acids like LEU [276], ALA [294], VAL [296], ARG [69] and SER [241].

Diosgenin and AMPK shows a stronger interaction with Akyl and amino acid like LEU [276], VAL [296].

Gitogenin interacted with AMPK via Pi-Alkyl linkage at VAL [296] PHE [272] PHE [243] and GLY [67]

Tigogenin and AMPK interreacted via VAL [296], LEU [276] and ALA [294] only.

Rutin shows a very strong interaction via VAL [29], LYS [26] , GLY [83] MET [84], THR [86], ASP [89] THR [88] HIS [150] and ILE [149] through various conventional non covalent Pi-Alkyls between the ligands and the amino acids. However, rutin disobey the Lipinski rule and

it could not cross the blood brain barrier. This makes its un suitable for drug likeness.

Finally under the section of activation of the central longevity protein, Neotigenin shows poor bond linkages. Only VAL [296] forms alkly bond with ligand

From the inhibition activities of mTOR, the best ten (10) inhibitors are reported with mTOR complex 4JSV and FKBP (4TW6). 4JSV is a complex of FKBP and mTOR while 4TW6 was docked with the ligands alone. It was reported that FKBP interact with rapalogs to form FKBP-Rapamycin binary complex that interacts with mTOR.

Tigogenin Inhibits mToR by interacting with LEU [1936], PRO [1975], PRO [2229] ILE [2228] the interaction between carpeine with highest binding score was between ARG [2224], GLY [2200], GLY [2142] PRO [1940]. Conventionally with, alkyl bond and hydrogen bond.

Sarsapogenin interacted with mToR via amino acid like PRO [1975], ILE [1939], PRO [1940], LEU [1900] alkyl-ligand bond formation.

Spirostanol interaction with 4JSV though amino acids like TYR [2225], ILE [2237] VAL [2240] and TRP [2239] via hydrogen bond formation, Pi-Alklyl bond.

Diosgenin Inhibits mTOR by interacting with Amino acids like LEU [1900] AND PRO [1975] via alkyl to ligand interaction.

Gitogenin interaction's with 4TW6 is by Donor-Donor and C-H conventional interaction Neotitogen and interaction reveal only LYS [66] interaction and C-H conventional sarsapogenin interact wth 4TW6 through PHE [77], ASP [68]. Here existed Pi-Alkyl and C-C interaction.

Spirostanol and 4TW6 interaction shows that PHE [130], LEU [128] ILE [122] interacted with the ligand and Pi-alkyl group interaction was observed.

Diosgenin and 4TW6 protein interaction shows that amino acids like LYS [88], ASP [91], ALA [95] were involved in the interaction. C-C interaction was observed as well as Pi-Alkly interaction.

Conclusion

It can be concluded that *T. foenum-graecum* contains some phytochemicals which are capable of activating the AMPK protein better than seglumet which is an analog of metformin presently under trial for prescription as an anti -ageing drugs. *T.Foenumas* also contains phytochemicals that can inhibit the mToR proteins or FKBP, better that rapamycin. It is hereby recommended that this result can be subjected to in vivo study for validation.

References

1. Adams, J. M., & White, M. (2004). Biological ageing: a fundamental, biological link between socio-economic status and health?. *The European Journal of Public Health*, *14*(3), 331-334.
2. Carling, D. (2004). The AMP-activated protein kinase cascade–a unifying system for energy control. *Trends in biochemical sciences*, *29*(1), 18-24.

3. Carling, D., Mayer, F. V., Sanders, M. J., & Gamblin, S. J. (2011). AMP-activated protein kinase: nature's energy sensor. *Nature chemical biology*, *7*(8), 512-518.

4. Eaknai, W., Bunwatcharaphansakun, P., Phungbun, C., Jantimaporn, A., Chaisri, S., Boonrungsiman, S., ... & Khongkow, M. (2022). Ethanolic fenugreek extract: its molecular mechanisms against skin aging and the enhanced functions by nanoencapsulation. *Pharmaceuticals*, *15*(2), 254.

5. Harman, D. (1992). Free radical theory of aging. *Mutation Research/DNAging*, *275*(3-6), 257-266.

6. Hiramatsu, M., Ninomiya, H., Inamura, K., Nomura, K., Takeuchi, K., Satoh, Y., ... & Ishikawa, Y. (2010). Activation status of receptor tyrosine kinase downstream pathways in primary lung adenocarcinoma with reference of KRAS and EGFR mutations. *Lung Cancer*, *70*(1), 94-102.

7. Liu, J., Chen, D., Liu, P., He, M., Li, J., Li, J., & Hu, L. (2014). Discovery, synthesis, and structure–activity relationships of 20 (S)-protopanaxadiol (PPD) derivatives as a novel class of AMPKα2β1γ1 activators. *European Journal of Medicinal Chemistry*, *79*, 340-349.

8. Ma, X. M., & Blenis, J. (2009). Molecular mechanisms of mTOR-mediated translational control. *Nature reviews Molecular cell biology*, *10*(5), 307-318.

9. Morath, C., Arns, W., Schwenger, V., Mehrabi, A., Fonouni, H., Schmidt, J., & Zeier, M. (2007). Sirolimus in renal transplantation. *Nephrology Dialysis Transplantation*, *22*(suppl_8), viii61-viii65.

10. Mendoza, N., & Silva, E. M. E. (2018). Introduction to phytochemicals: secondary metabolites from plants with active principles for pharmacological importance. *Phytochemicals: Source of antioxidants and role in disease prevention*, *25*, 1-5.

11. Rastogi, R. P. (2015). UV-induced oxidative stress in cyanobacteria: how life is able to survive?. *Biochemistry & Analytical Biochemistry*, *4*(02), 173.

12. Sreenivasan Snima, K., Pillai, P., Mary Cherian, A., V. Nair, S., & Lakshmanan, V. K. (2014). Anti-diabetic drug metformin: challenges and perspectives for cancer therapy. *Current cancer drug targets*, *14*(8), 727-736.

13. Sabers, C. J., Martin, M. M., Brunn, G. J., Williams, J. M., Dumont, F. J., Wiederrecht, G., & Abraham, R. T. (1995). Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells (∗). *Journal of Biological Chemistry*, *270*(2), 815-822.

14. Xu, Q., & Si, L. Y. (2010). Protective effects of AMP-activated protein kinase in the cardiovascular system. *Journal of cellular and molecular medicine*, *14*(11), 2604-2613.

15. Viollet, B., Foretz, M., & Schlattner, U. (2014). Bypassing AMPK phosphorylation. *Chemistry & Biology*, *21*(5), 567-569.

16. Wani, S. A., & Kumar, P. (2018). Fenugreek: A review on its nutraceutical properties and utilization in various food products. *Journal of the Saudi Society of Agricultural Sciences*, *17*(2), 97-106.

17. Yu, M., Zhang, H., Wang, B., Zhang, Y., Zheng, X., Shao, B., ... & Jin, K. (2021). Key signaling pathways in aging and potential interventions for healthy aging. *Cells*, *10*(3), 660.

18. Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P., & Li, H. B. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*, *20*(12), 21138-21156.